

# Kinetics of Plasma Viremia and Soluble Nonstructural Protein 1 Concentrations in Dengue: Differential Effects According to Serotype and Immune Status

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**We describe the magnitude and kinetics of plasma viremia and nonstructural protein 1 (sNS1) levels in sequential samples from 167 children with acute dengue, enrolled early in a community study in Vietnam. All children recovered fully, and only 5 required hospitalization. Among those with dengue virus type 1 (DENV-1), plasma viremia was significantly greater in primary (49) than secondary (44) infections and took longer to resolve. In primary DENV-2 and 3 infections, viremia was significantly lower than among primary DENV-1 infections. Concentrations of sNS1 were significantly higher for DENV-1 than for DENV-2 after adjusting for viremia, with marked differences in the kinetic profiles between primary and secondary infections. Secondary infection and higher viremia were independent predictors of more severe thrombocytopenia, and higher viremia was associated with a small increase in hemoconcentration. Our findings identify clear serotype and immune-status related effects on the dynamics of dengue viremia and sNS1 responses, together with associations with important clinical parameters.**

Dengue is an arboviral disease caused by infection with 1 of 4 related viral serotypes (DENV1–4) [1]. It is a major threat to global health, with at least 3 billion persons at risk of infection and ~40 million symptomatic episodes estimated to occur annually [2]. A wide spectrum of disease manifestations is seen, ranging from inapparent infection to severe and potentially fatal disease [1]. Endothelial dysfunction is the characteristic feature of severe disease; increased vascular permeability

results in hypovolemic shock, usually accompanied by deranged hemostasis and significant thrombocytopenia. Neither vaccines nor specific therapies are currently available, and management relies on provision of good supportive care for symptomatic cases.

A variety of viral and host factors are thought to contribute to pathogenesis [3]. Although severe disease may occasionally be seen during primary DENV infection, particularly in infants [4, 5], complications are usually described in association with sequential or secondary infections [6, 7]. Antibody-dependent enhancement (ADE) is thought to underlie this phenomenon [8–10], although altered cellular immune responses have also been implicated [11]. According to the ADE hypothesis, nonneutralizing virion-binding antibodies acquired passively or during an earlier infection bind to the new virus and facilitate uptake by Fc receptor-bearing cells, amplifying the infection, and the increased viral burden drives an immunopathogenic cascade that alters microvascular function in an undefined way. However, data on plasma viremia in primary versus secondary

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infection and in relation to disease severity remain limited and inconsistent. Early reports indicated that the duration and magnitude of viremia did not vary significantly with immune status, with some studies demonstrating higher viremia in primary infections [12–15]. Subsequent studies showed associations between increased clinical severity, higher viremia, and secondary infections among hospitalized Asian children [16, 17]. Other groups, however, found associations between viremia and clinical severity without evidence of a relationship with immune status [18–20]. However, the number of patients with sufficient early assessments to allow detailed examination of viremia kinetics has been limited, with the focus primarily on hospitalized patients.

Dengue nonstructural protein 1 (sNS1) is ~46 kDa glycoprotein secreted by infected mammalian cells, although a biological role in disease pathogenesis remains unclear [21, 22]. High levels of circulating sNS1 have been found during acute infections, and it has been proposed as a prognostic marker for severe disease; however, there are few data describing the period of the response or relationships to serotype or immune status [23–25].

The aim of this study was to describe the kinetics of plasma viremia and sNS1 responses in children with dengue who were recruited early in the community and monitored carefully throughout the course of their illness.

## MATERIALS AND METHODS

### Study Population and Clinical Methods

Recruitment took place at a community clinic in Ho Chi Minh City run by an experienced dengue physician based at the Hospital for Tropical Diseases (HTD). Patients aged 5–15 years who presented with suspected dengue and fever for <72 h were eligible for enrollment, provided a parent gave written informed consent and children aged >12 years gave assent. Ethical approval was obtained from the Ethical Committee of HTD and the Oxford Tropical Research Ethics Committee. Patients were seen daily until afebrile for 2 consecutive days, with standardized clinical information recorded and a 1-mL venous blood sample obtained at each visit. The physician was responsible for all management decisions and could admit to HTD at any time. All inpatients were assessed daily using the same protocol. Families were also given a card allowing direct access to the emergency department at HTD. Patients were invited to attend for review 3–4 weeks from illness onset.

Illness day 1 was defined as the day of reported fever onset. The day of defervescence was defined as the illness day on which the oral temperature first decreased to  $\leq 37.5^{\circ}\text{C}$ , with no subsequent increase. Fever day was defined in accordance with earlier publications, taking the day of defervescence as fever day 0 [16, 26]. The following clinical outcomes were summarized from the daily assessments: the platelet nadir, the presence or absence of skin and/or mucosal bleeding, and the percentage increase in hematocrit comparing the maximum value between

days 3–8 of illness with a baseline value taken as the lowest result obtained on or before illness day 2 or after day 14.

### Virological and Serological Assays

Dengue diagnostic capture IgM and IgG enzyme-linked immunosorbent assays (ELISAs) were performed as described elsewhere, with use of paired enrollment and convalescent specimens and reagents provided by Venture Technologies [24, 27]. To define immune status, an indirect ELISA to pooled recombinant E proteins of the 4 DENV serotypes (Hawaii Biotech) was used. In brief, after blocking with 3% Bovine Serum Albumin, 1:50 dilutions of patient or healthy donor plasma samples in phosphate-buffered saline were incubated in coated and uncoated wells for 2 h, followed by horseradish peroxidase-conjugated goat anti-human IgG for 2 h, then the substrate tetramethylbenzidine, and the optical density (OD) was read at 450 nm. A cut-off 3 times the mean OD of healthy donor plasma, after subtraction of the OD in uncoated wells, was used; a sample to cut-off ratio  $< 0.8$  was considered to be negative, and a ratio  $> 1.2$  was considered to be positive. A negative indirect ELISA result in the first 72 h with no increase in dengue-reactive IgG by day 7 defined a primary infection, and a positive indirect ELISA result within 72 h and/or an equivocal response, with a clear increase in dengue-reactive IgG greater than the IgM response by day 7, defined a secondary infection.

DENV detection by reverse-transcription polymerase chain reaction (RT-PCR) in enrollment plasma samples was performed using the method described by Shu et al [28]. Subsequently, daily DENV load was measured using an internally controlled, serotype-specific, real-time RT-PCR assay, as described elsewhere [29]. The assay is equally sensitive for DENV-1, -2, and -3, as demonstrated by the equivalent detection of standardized amounts of in vitro transcribed RNA molecules that correspond to the respective target sequence in each serotype. Serial plasma samples from each patient were assayed on the same day and grouped by serotype, and all assays were done by one person blinded to the clinical outcome. The day of viral clearance was defined as the illness day on which the RT-PCR first became negative and remained negative.

Qualitative sNS1 assays were performed using Biorad Platelia Dengue NS1 Antigen kits, according to the manufacturer's instructions. A quantitative assay was developed using the same kits and using affinity-purified recombinant baculovirus derived DENV-2 sNS1 to establish standard curves, with serial dilutions of sNS1 from 2 ng/mL to 0.0625 ng/mL, run in duplicate on each plate. All serial samples, diluted up to 1:10,000, from any patient positive on the qualitative sNS1 assay were run on the same plate; the limit of detection was 0.125 ng/mL.

### Statistical Methods

For viremia and sNS1 concentrations, the illness day 3 value, the proportion of patients with undetectable measurements at illness day 6, and the area under the curve (AUC) of the serial

measurements obtained during days 3–6 (estimated by the trapezoidal rule) were used as summary measures describing the period when almost all patients were observed. In additional sensitivity analyses, we examined viremia and sNS1 concentrations on fever day -2, the proportion of patients with undetectable measurements on fever day 0, and for viremia only, the maximum observed value and time to viral clearance (patients without documented clearance were treated as censored on the last day of assessment). Finally, when possible, we determined the peak viral load (ie, if there were documented lower viral loads on the days before and after the maximum observed value).

To compare viremia kinetics, we predefined the following contrasts to be of primary importance: pair-wise comparisons of serotypes in the subgroups of patients with primary and secondary infection and comparisons of primary versus secondary infection within dengue serotypes. Comparisons of log-transformed viral load measurements were based on linear regression models, comparisons of detectable rates on a Bayesian version of logistic regression [30], and comparisons of time to viral clearance on a Cox regression model. In all cases, we included dummy covariates for each serotype immune status combination and tested for significance of the prespecified contrasts. We corrected for multiple comparisons with use of a single-step approach based on the joint asymptotic multivariate distribution of the contrasts [31]. Plasma sNS1 kinetics were analyzed similarly, except that we excluded patients infected with DENV-3 because of the limited available data.

Clinical outcomes were modeled with linear or logistic regression models, as appropriate. The following covariates were jointly included in the models:  $\log_{10}$  dengue viremia on illness day 3, serotype, and immune status. For laboratory outcomes, we also added the baseline value of the respective parameter as a covariate. Alternative models that replaced  $\log_{10}$  dengue viremia with  $\log_{10}$  sNS1 were also examined. Finally, we tested for an interaction between immune status and serotype and added it to the model if significant.

A small number of children were not followed up until illness day 6, and we used a single random imputation for missing viral load and sNS1 values from these patients. All analyses were performed using R, version 2.9.1 [32], and the R packages multcomp, arm, and mice.

## RESULTS

### Characteristics of the Study Population

From June 2006 through March 2008, 301 children were enrolled in the study; 203 (67%) had dengue confirmed by RT-PCR, and 8 (3%) had serological evidence of infection. All dengue diagnostics were negative in 80 children (27%), and the results were indeterminate for 10 (3%). For the present study, we included 167 (82%) of the RT-PCR-positive patients; children were excluded because they had unknown or mixed ( $n = 6$ ) or DENV-4

( $n = 2$ ) infections, they entered the cohort late ( $n = 9$ ), or their immune status was indeterminate ( $n = 19$ ). Each child attended a median of 6 daily visits (interquartile range [IQR], 5–6 daily visits) during the acute illness, 11 (7%) of 167 stopped review before day 6 (but all were afebrile at this time), and 8 had already cleared their viremia. One hundred fifty-three (92%) of 167 attended the follow-up visit. Daily plasma viral load measurements were performed for all participants, and daily sNS1 concentrations were measured for all participants enrolled from June 2006 through September 2007 (82 [49%] of 167).

The main demographic and clinical characteristics of the study population are summarized in Table 1. The median age of the children was 11 years (IQR, 9–13 years), and 83 (50%) were female. DENV-1 was the predominant serotype identified; 93 (56%) of the children were infected with DENV-1, 36 (22%) with DENV-2, and 38 (23%) with DENV-3. The proportion with primary infections was lower for DENV-2 than for the other serotypes ( $P = .04$ , by  $\chi^2$  test): 53% for DENV-1, 28% for DENV-2, and 45% for DENV-3. Time to defervescence was generally shorter for secondary than for primary infections (Cox regression hazard ratio, 1.46; 95% confidence interval [CI], 1.06–2.02;  $P = .02$ ), with no evidence of a serotype effect ( $P = .15$ , likelihood ratio test). Almost all children (155 [93%] of 167) received some intravenous fluid replacement as outpatients, primarily maintenance infusions given during the early febrile phase; a similar pattern of fluid intervention was seen among children without dengue, with 73 (91%) of 80 receiving infusions. Two children were reviewed in the emergency department but not admitted, and 5 children required hospitalization. Two of the hospitalized children developed dengue shock syndrome but recovered promptly after standard fluid resuscitation; both had secondary infections (DENV-2 and DENV-3), and the remaining 3 hospitalized children included 2 primary infections and 1 secondary infection (all DENV-1).

### Plasma Viremia Kinetics

Key summaries of plasma viremia and sNS1 concentrations are presented in Table 2, with the kinetics of these parameters shown in Figures 1 and 2. Additional summary information in relation to fever day, plus data on time to viral clearance and maximum viral load, are displayed in Supplementary Table 1 and Supplementary Figure 1. AUCs and illness day 3 measurements correlated strongly with each other (partial correlation after controlling for immune status and serotype, 0.96 for viremia and 0.98 for sNS1 concentration). There was also a statistically significant association between the viremia and sNS1 measurements (partial correlation, 0.58 for AUCs and 0.56 for day 3 measurements; both  $P < .001$ ).

Patients with DENV-1 primary infections had significantly higher viral loads, expressed in terms of illness day 3 measurements and AUCs, compared with patients with secondary DENV-1 infections (both  $P < 0.02$ ). Maximum observed viral loads were

**Table 1. Demographic and Clinical Characteristics of the 167 Children with Dengue Included in the Study**

	DENV-1		DENV-2		DENV-3	
	Primary (n = 49)	Secondary (n = 44)	Primary (n = 10)	Secondary (n = 26)	Primary (n = 17)	Secondary (n = 21)
Demographics						
Age, years	11	12	10	12	10	12
Median (IQR)	(8-13)	(9-13)	(6-11)	(11-14)	(7-13)	(10-13)
Sex, female						
n (%)	25 (51%)	15 (34%)	6 (60%)	15 (58%)	11 (65%)	11 (52%)
Day of illness at enrolment						
Day 1, n (%)	12 (24%)	8 (18%)	4 (40%)	2 (8%)	3 (18%)	2 (10%)
Day 2, n (%)	21 (43%)	19 (43%)	5 (50%)	15 (58%)	12 (71%)	7 (33%)
Day 3, n (%)	16 (33%)	17 (39%)	1 (10%)	9 (35%)	2 (12%)	12 (57%)
Days of sequential follow-up						
Median (IQR)	6 (5-7)	6 (5-6)	6 (5-6)	5 (5-6)	6 (5-6)	6 (4-6)
Attended convalescent review						
n (%)	46 (94%)	37 (84%)	10 (100%)	26 (100%)	15 (88%)	19 (90%)
Clinical outcomes						
Platelet nadir, cells/ $\mu$ L	110,000	96,400	155,000	81,000	135,000	85,600
Median (IQR)	(83,900-143,000)	(67,525-131,000)	(130,500-165,750)	(53,075-133,500)	(84,000-149,000)	(71,000-114,000)
Day of illness of platelet nadir						
Median (IQR)	6 (5-7)	5 (5-6)	5 (4-7)	5 (4-6)	6 (4-6)	5 (5-6)
Bleeding						
Skin only, n (%)	29 (59%)	24 (55%)	7 (70%)	13 (50%)	12 (71%)	12 (57%)
Mucosal, n (%)	4 (8%)	3 (7%)	0 (0%)	3 (12%)	0 (0%)	1 (5%)
Percentage hemoconcentration						
Median (IQR)	14 (7-22)	11 (6-19)	10 (6-14)	16 (12-26)	8 (3-12)	14 (5-19)
Day of defervescence						
Median (IQR)	6 (5-6)	5 (5-6)	5 (4-6)	5 (4-5)	6 (5-6)	5 (5-6)

almost 1  $\log_{10}$  copies/mL higher for primary compared with secondary DENV-1 infections (median viral load, 8.78 vs 7.83  $\log_{10}$  copies/mL, comparison adjusted for enrollment day;  $P = .006$ ). Peak viremia could be identified in 17 primary and 20 secondary DENV-1 infections, and median peak values in the 2 groups were 9.37 and 8.42  $\log_{10}$  copies/mL, respectively. Moreover, patients with primary DENV-1 infection were viremic for significantly longer than were patients with secondary DENV-1 infections (median, 7 vs 5 days to viral clearance;  $P < .001$ ) and were more likely to still be viremic at defervescence (31% vs 66% below the limit of detection on fever day 0;  $P = .01$ ). In relation to fever day, we found very similar primary and secondary plasma viral loads on fever day -2. Results for comparisons on earlier fever days -3 and -4 (data not shown) suggest higher viral loads in the primary group, but the number of observations available at these times was small.

Comparisons between serotypes revealed that viral loads in primary infections were significantly lower for DENV-2 and DENV-3 ( $P < .01$ , for each serotype comparison with DENV-1, both for illness day 3 and AUCs), with the median day 3 viremia measurements 2.5 and 1.6  $\log_{10}$  copies/mL lower for these

serotypes than for DENV-1. The results suggest that higher plasma viral load occurs in patients with secondary DENV-2 infection and, possibly, DENV-3 infection, compared with primary infection; however, the number of infections due to these serotypes was small, and no statistically significant differences were observed for any of the predefined analyses. Viremia was undetectable in the majority of patients with these serotypes by illness day 6, with no differences in the proportion of patients with primary or secondary infections testing negative at this time or on the day of defervescence.

#### Plasma sNS1 Kinetics

The magnitude and kinetics of the sNS1 responses also varied markedly by serotype and immune-status. Patients with primary DENV-1 infection had high plasma concentrations from the early febrile period persisting at high levels throughout the illness, and in secondary DENV-1 infections the plasma levels decreased rapidly from day 4–5 onward. Plasma sNS1 levels on illness day 3 were markedly lower in patients with DENV-2 infection, compared with patients with DENV-1 infection ( $P \leq .001$ , for comparisons in both primary and

**Table 2. Summary and Comparison of Plasma Viremia Levels and sNS1 Concentrations Between Illness Days 3–6 by Dengue Serotype and Immune Status**

	DENV-1		DENV-2		DENV-3	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
<b>Dengue viremia</b>						
Number of observations	49	44	10	26	17	21
Day 3 measurement <sup>a</sup>	8.34	7.71	5.85	7.54	6.77	7.62
Median (IQR) [log <sub>10</sub> -copies/ml]	(7.56–8.96)	(6.17–8.41)	(4.43–7.55)	(6.23–8.26)	(5.80–7.56)	(6.15–7.97)
AUC days 3–6 <sup>b</sup>	8.07	7.48	5.76	7.32	6.63	7.45
Median (IQR) [log <sub>10</sub> -copies/ml]	(7.35–8.89)	(6.16–8.28)	(4.69–7.35)	(6.40–8.03)	(5.50–7.51)	(6.14–8.17)
Day 6 measurement <sup>c</sup>	20 (41%)	40 (91%)	9 (90%)	24 (92%)	13 (76%)	16 (76%)
n (%) below the detection limit						
<b>sNS1 Plasma concentration</b>						
Number of observations	26	23	9	18	5	1
Day 3 measurement <sup>d</sup>	2,569	390	4	10	4,443	9,408
Median (IQR) [ng/ml]	(1,639 - 4,734)	(85 - 1,722)	(2 - 5)	(ND - 101)	(2,804 - 4,990)	(-, -)
AUC days 3–6 <sup>e</sup>	9,310	225	11	8	14,654	13,751
Median (IQR) [ng/ml]	(4,478 - 15,219)	(43 - 2,699)	(4 - 14)	(ND - 163)	(7,18 - 15,051)	(-, -)
Day 6 measurement <sup>f</sup>	0 (0%)	20 (90%)	3 (33%)	16 (89%)	0 (0%)	1 (100%)
n (%) below the detection limit						

**NOTE.** ND indicates below the detection limit for the assay. *P* values adjusted for multiple comparisons (D1.P=DENV-1, primary infection etc.; only DENV-1 and DENV-2 are included in comparisons of sNS1 in plasma):

- <sup>a</sup> Plasma viremia – day 3: D1.P – D2.P (*P* < .001), D1.P – D3.P (*P* = .008), D1.P – D1.S (*P* = .01), all other adjusted *P* > .4
- <sup>b</sup> Plasma viremia – AUC: D1.P – D2.P (*P* < .001), D1.P – D3.P (*P* = .004), D1.P – D1.S (*P* = .02), all other adjusted *P* > .35
- <sup>c</sup> Plasma viremia – detectable on day 6: D1.P – D2.P (*P* = .13), D1.P – D3.P (*P* = .18), D1.P – D1.S (*P* < .001), all other adjusted *P* > .5
- <sup>d</sup> sNS1 in plasma – day 3: D1.P – D2.P (*P* < .001), D1.S – D2.S (*P* = .001), D1.P – D1.S (*P* = .02), D2.P – D2.S (*P* = 1.00)
- <sup>e</sup> sNS1 in plasma – AUC: D1.P – D2.P (*P* < .001), D1.S – D2.S (*P* = .002), D1.P – D1.S (*P* < .001), D2.P – D2.S (*P* = .94)
- <sup>f</sup> sNS1 in plasma – detectable on day 6: D1.P – D2.P (*P* = .10), D1.S – D2.S (*P* = 1.00), D1.P – D1.S (*P* < .001), D2.P – D2.S (*P* = .02)

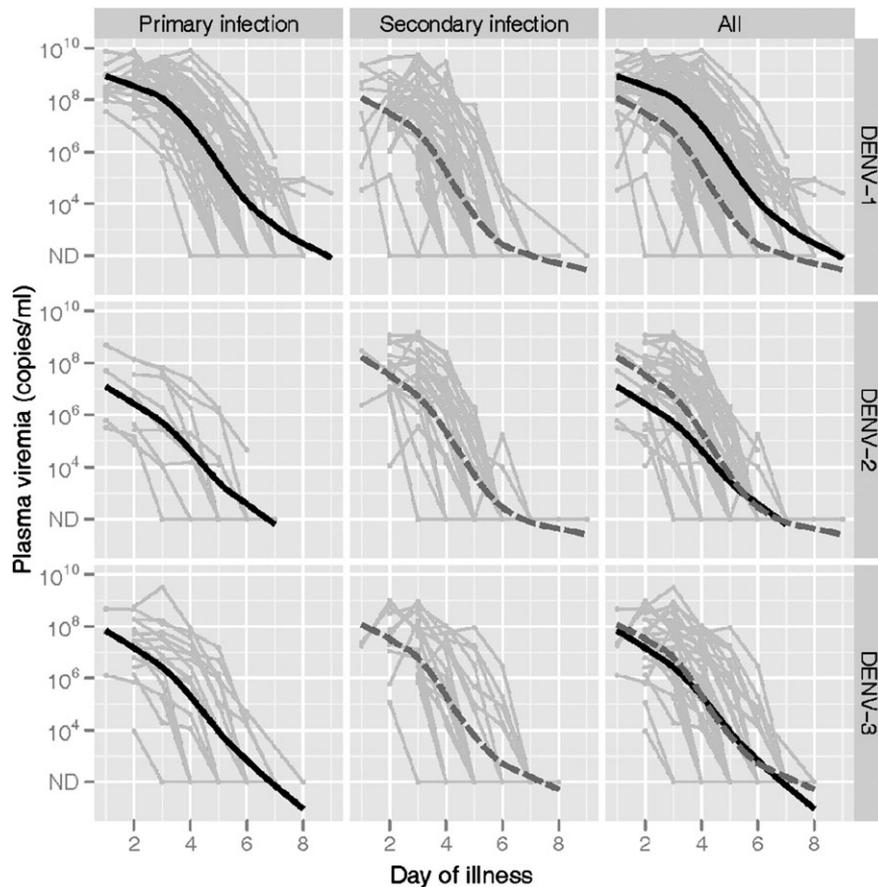
secondary infections), and this remained unchanged after adjustment for log<sub>10</sub> viral load on the same day (both *P* < .006). One third of all DENV-2–infected patients were consistently sNS1 negative throughout the illness course; although 7 of 9 had secondary infections, the remaining 2 had primary infections. Patients with secondary DENV-1 and DENV-2 infections were significantly more likely to have undetectable sNS1 levels by illness day 6, compared with patients with primary infections due to the same serotype (both *P* ≤ .02). Data were available for only 6 patients with DENV-3 infection, but the results suggest a pattern similar to that for patients with DENV-1 infection.

### Associations with Clinical Outcomes

Data describing the main clinical outcomes are presented in Table 1. Median platelet counts were consistently lower in secondary infections during illness days 3–7 for all 3 serotypes (Figure 3). Linear regression models showed that secondary infection (estimate, -32,680 cells/μL; 95% CI, -45,796 to -19,564; *P* < .001) and higher viral load on illness day 3 (estimate, -11,696 cells/μL per +1log<sub>10</sub> copies/mL; 95% CI, -15,373 to -8019; *P* < .001) are independent predictors of a lower platelet nadir, with no evidence that serotype plays a role (*F* test, *P* = .53) or of an interaction between immune status and viremia (*F* test, *P* = .24). In addition,

higher plasma sNS1 concentrations were significantly associated with a lower platelet nadir after adjusting for serotype and immune status (*P* < .001), but the association was only borderline significant when entered jointly with viremia (*P* = .07). Minor clinical bleeding was noted in 61%–71% of the patient subgroups (Table 1), but no association with serotype or immune status was apparent.

The severity of vascular leakage was assessed in terms of maximum percentage hemoconcentration. In the regression model, a significant interaction between serotype and immune status was apparent (*F* test, *P* = .03); thus, greater hemoconcentration was observed in secondary than in primary infections due to DENV-2 (*P* = .06) and DENV-3 (*P* = .01) but not DENV-1 infections (*P* = .65). In fact, among the patients with DENV-1 infection, a greater proportion of those with primary (16 [33%] of 49) than with secondary infections (11 [25%] of 44) developed hemoconcentration ≥20% relative to baseline. The severity of hemoconcentration in patients with primary DENV-3 infection was also significantly less than that seen in primary DENV-1 infection (*P* = .008). Results obtained after adjustment for the cumulative fluid volume per kilogram body weight were very similar, with no evidence for any effect of immune status for DENV-1 infection. Finally, higher viral load on illness day 3 was an independent predictor of higher



**Figure 1.** Dengue viremia kinetics in 167 children with dengue, by serotype and immune status, presented according to illness day. Gray lines correspond to individual children, and the thick lines correspond to loess scatter plot smoothers.

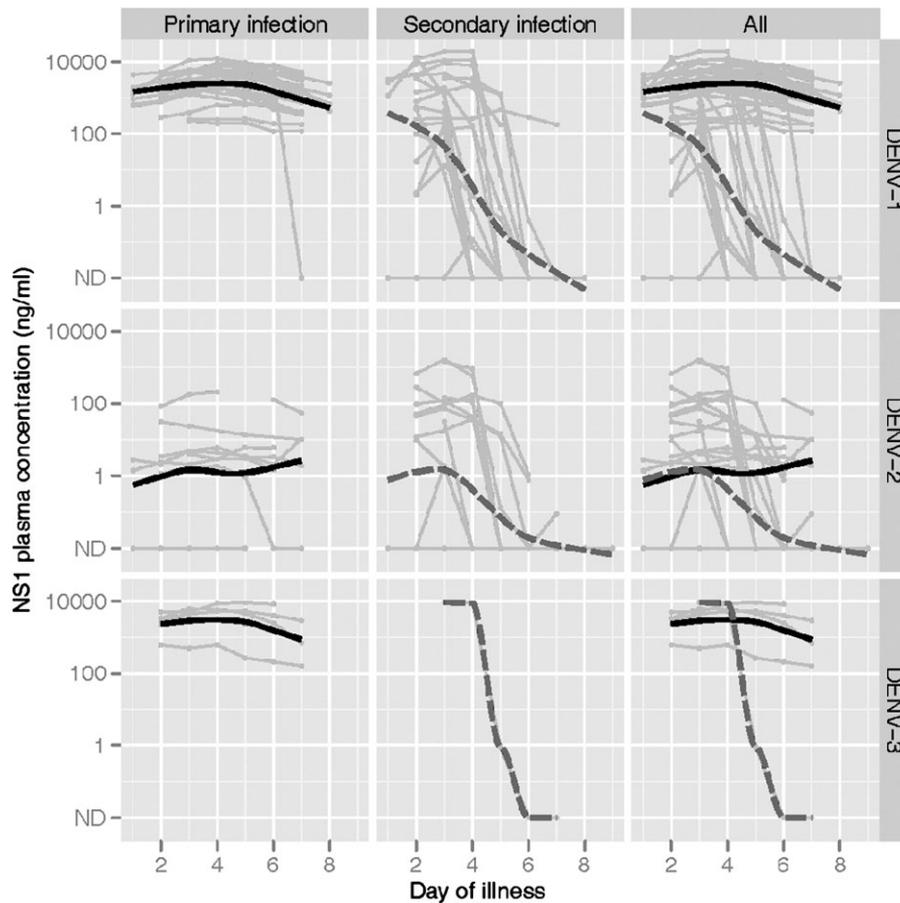
hemoconcentration, although the effect size was small (estimate, +1.2% per +1log<sub>10</sub> copies/mL; 95% CI, .15–2.26; *P* = .03), and no association was observed between hemoconcentration and sNS1 level (*P* = .23).

## DISCUSSION

In this prospective observational study, we describe the magnitude and kinetics of plasma viremia and sNS1 levels in 167 children enrolled in a community dengue study. Early recruitment facilitated viral identification in virtually all cases, and careful follow-up allowed detailed assessment of the responses over time.

In the 93 children infected with DENV-1, plasma viremia, assessed by a variety of methods, was significantly greater in primary than in secondary infections and took longer to resolve. By contrast, the measurable burden in primary DENV-2 and DENV-3 infections was significantly lower than that in primary DENV-1 infection, with a suggestion of increased viral load at least during secondary DENV-2 infection. All comparisons were predefined and adjusted for multiple testing, and thus, the results are quite conservative.

The characteristics of the sNS1 responses also differed markedly across the groups. We found considerably higher sNS1 concentrations in DENV-1 infection than in DENV-2 infection, even after adjusting for viremia. Although the sNS1 kits used are reported to bind all serotypes similarly, the possibility of differential sensitivity for DENV-1 and DENV-2 cannot be excluded. However, although all viruses identified during this period were from the same lineage [33], there was no measurable sNS1 response in one-third of DENV-2-infected patients, including 2 with primary infections, indicating that, in DENV-2, there is also considerable variability in sNS1 responses. We also noted a rapid decrease in secondary but not primary infections from day 5 of illness, potentially consistent with immune complex formation. Most patients had mild disease and were treated in the community without development of complications, emphasizing the bias that may be introduced by focusing research on hospitalized patients. Although previous studies have suggested a relationship between sNS1 levels and disease severity, formal prospective evaluation across a range of health care settings should be performed to assess whether quantitative sNS1 measurements are actually useful for risk prediction.

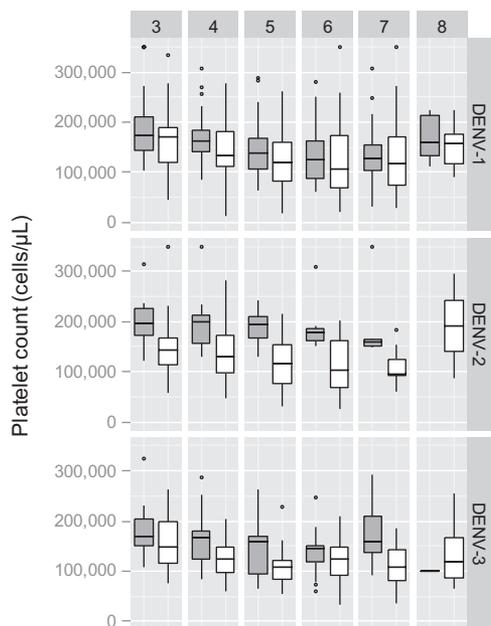


**Figure 2.** Plasma sNS1 concentration kinetics in 82 children with dengue, by serotype and immune status, presented according to illness day. Gray lines correspond to individual children, and the thick lines correspond to loess scatter plot smoothers. (Only one child with secondary DENV-3 was assessed.)

Caution is necessary in interpreting the significance of the findings with respect to immune enhancement. First, the results reflect concentrations detectable in plasma, but virus and/or protein might be sequestered in other tissues or be inaccessible in immune complexes and still retain the potential to influence disease pathogenesis. Second, despite a study design aimed at early recruitment, of necessity, we could only observe the responses after the onset of symptoms. In the presymptomatic phase of secondary infection, ADE may transiently boost infection, prompting a robust anamnestic immune response, such that the plasma viral load may already be decreasing by the time the patient becomes symptomatic. In primary infection, peak viremia may occur later, with the whole viremia-to-time curve shifted to the right. Leaving aside ADE, the study reveals that DENV-1 behaves differently from other serotypes, suggesting an intrinsic fitness advantage that allows development of high viral loads in the absence of pre-existing antibody, in contrast to DENV-2 and DENV-3, in which a heterologous immune response seems necessary to develop high viral loads. However, RT-PCR measures genome copies, comprising both infectious and noninfectious viral particles, and the relative proportions

may well vary between serotypes. Assessment of virus titers based on plaque assay methods should clarify whether DENV-1 is indeed associated with a higher infectious viral burden than other serotypes. Finally, although these results reflect the responses of the serotypes and genotypes circulating in Vietnam during the study period, further work is needed to determine whether the findings are generalizable to other settings. If confirmed, the implications for studies of transmission dynamics in different immune landscapes may be considerable.

With respect to clinical outcome, examination of relationships to individual markers of severity indicates that secondary infection and higher viral load were independent predictors of more severe thrombocytopenia. Higher viral load was also independently associated with a small effect on hemoconcentration; consistent with this finding, hemoconcentration of  $>20\%$  was more frequent in primary than in secondary DENV-1 infections. Previously, in a study of secondary DENV-3 infections, Libraty et al [17] reported associations between higher viral load and both hemoconcentration and thrombocytopenia; this study provides evidence that higher viral load also exerts an effect in primary infection and across different serotypes.



**Figure 3.** Boxplots of serial platelet counts in 167 children with dengue during illness days 3–8, by serotype. Gray boxplots correspond to children with primary infection, and white boxplots correspond to children with secondary infection. (Platelet values >350,000 cells/μL were truncated at that value in the display.)

In conclusion, we found that the infecting serotype and the host's primary or secondary immune status contribute to viremia and sNS1 profiles during dengue infection and influence various important clinical parameters. Many other host and viral determinants probably contribute, and because of the intrinsic variability in such biological factors, it is likely that future studies will require early enrollment of large numbers of patients if the relative contributions to pathogenesis are to be clearly elucidated. Such knowledge of relationships between viremia, viral biomarker kinetics, and clinical outcome will be invaluable for the design and conduct of therapeutic trials aimed at preventing and/or treating severe dengue and should also improve our understanding of pathogenesis and transmission dynamics.

### Supplementary Data

Supplementary data are available at <http://jid.oxfordjournals.org> online.

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